Optimized lipid nanoparticles for CRISPR/Cas9 delivery: advancing gene editing in lung tumor cells

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NEXT-GENERATION DELIVERY INNOVATIONS

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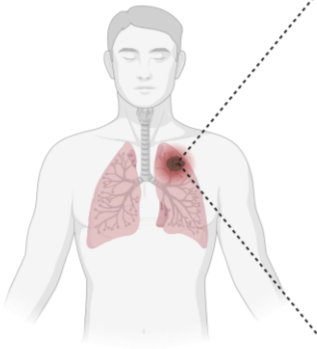
Lung cancer



- A leading cause of cancer-related deaths worldwide
- Diagnosis at a later stage ⟿ hard to implement treatment ⟿ low survival rate

KRAS mutations:

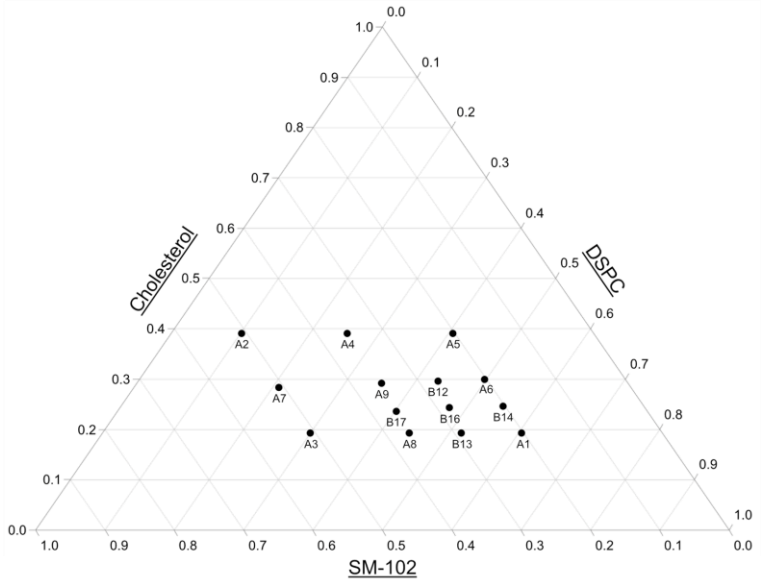
- 15–30% of lung cancer cases
- Limited effectiveness and resistance to standard therapies (tyrosine kinase inhibitors) ⟿ poor prognosis and reduced survival
- Undruggable target ⟿ Sotorasib and Adagrasib

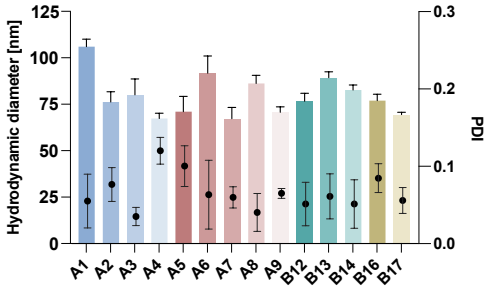
(Siegel et al., 2018; Dogan et al., 2012; Ferrer et al., 2018)

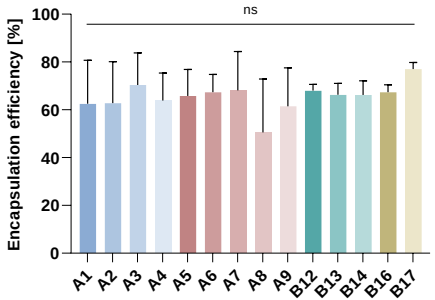
CRISPR/Cas9-LNPs for the precise editing of KRAS mutations

Optimization of mRNA-LNPs

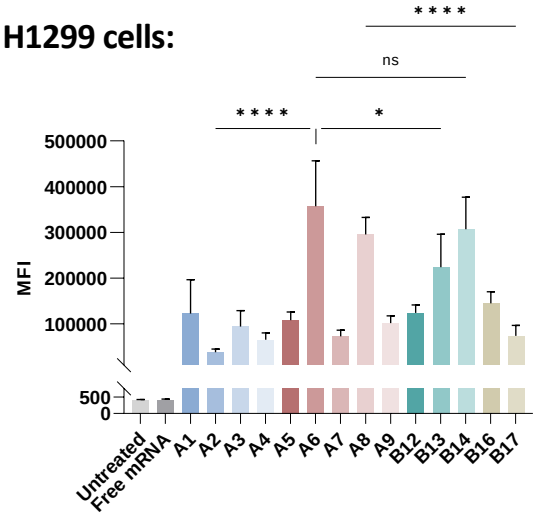
Screening lipid concentrations:







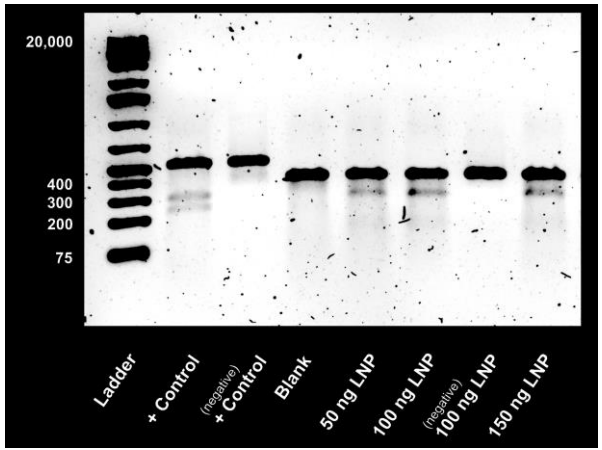
eGFP expression in H1299 cells:

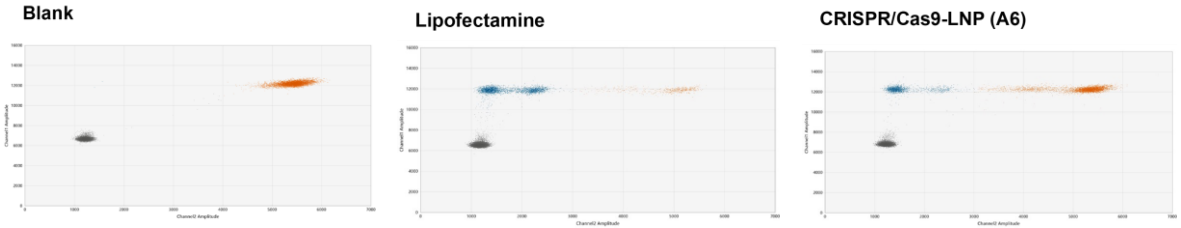


CRISPR/Cas9-LNPs

Gene editing efficiency of A6 in A549 cells

T7EI indel detection

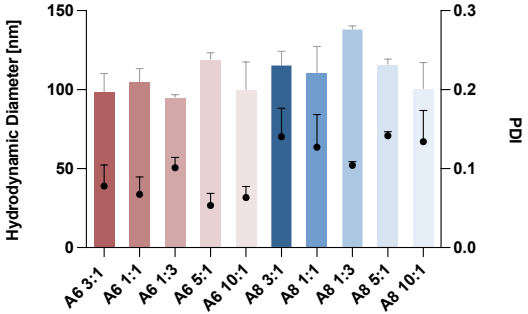


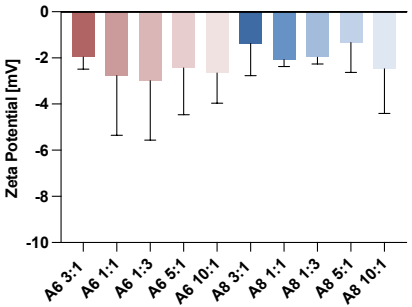


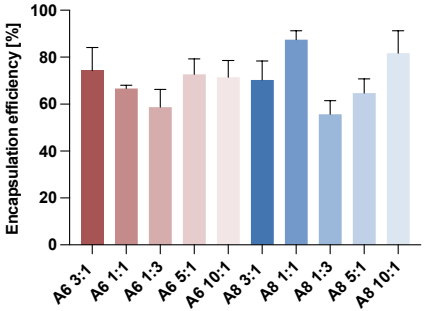
Sample	Gene editing efficiency (%)
Blank	0.04
Positive control	87.20
CRISPR/Cas9-LNP (A6)	35.50

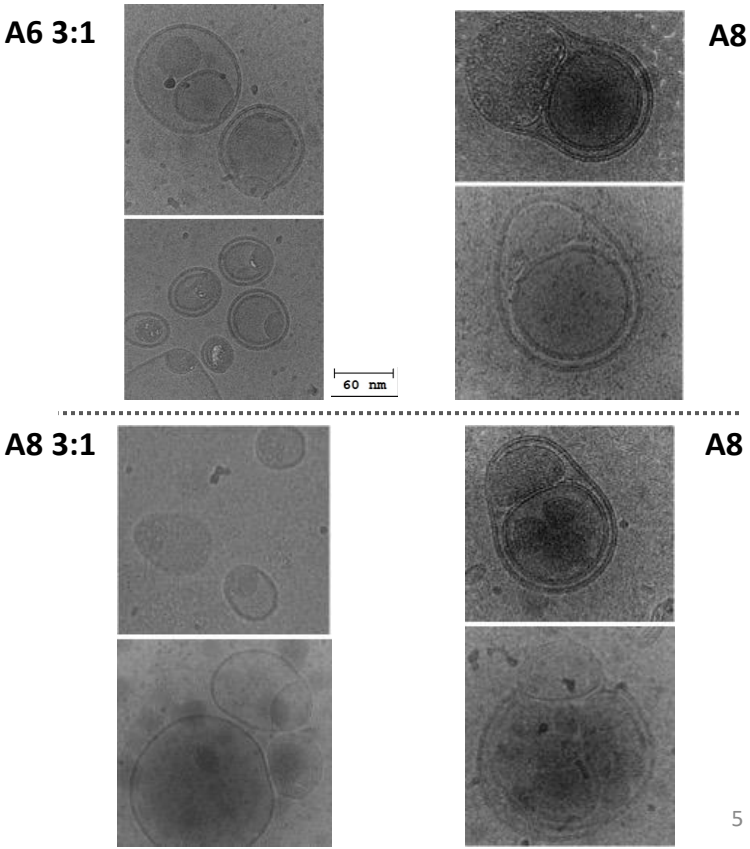
CRISPR/Cas9-LNPs

Optimization of mCas9:sgRNA w/w ratio:









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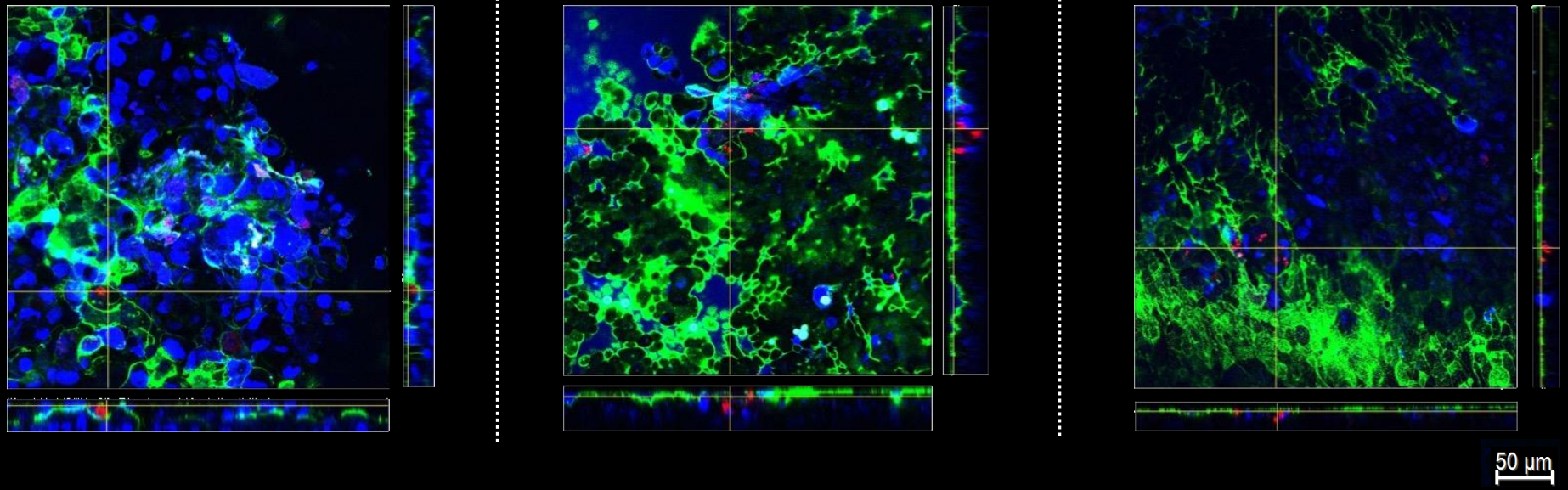
Mucus penetration assay

Free mCas9: sgRNA | **A6 3:1** | **A8 1:1**



Hoechst, nuclei
AlexaFluor488-labeled Wheat-Germ-Agglutinin, mucus
AlexaFluor647-labeled mRNA

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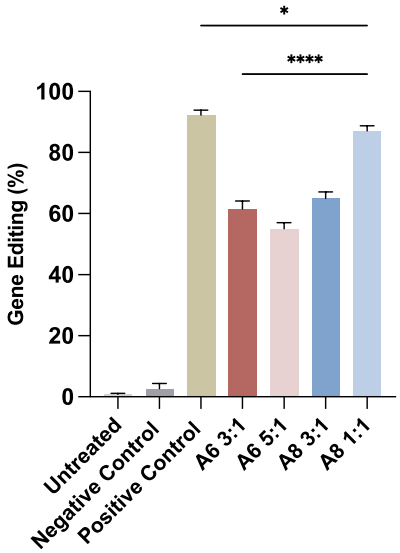
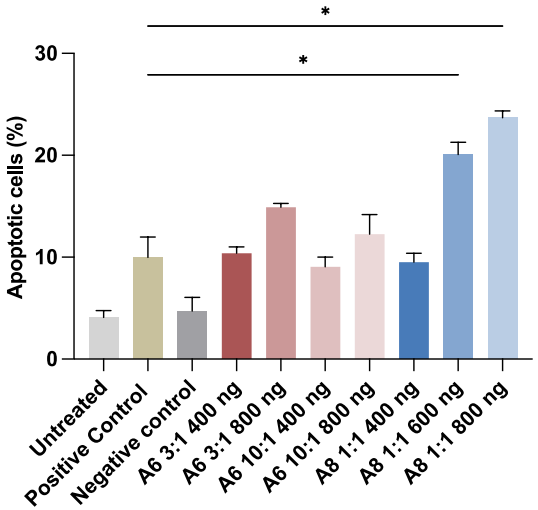
Optimization of mCas9:sgRNA w/w ratio

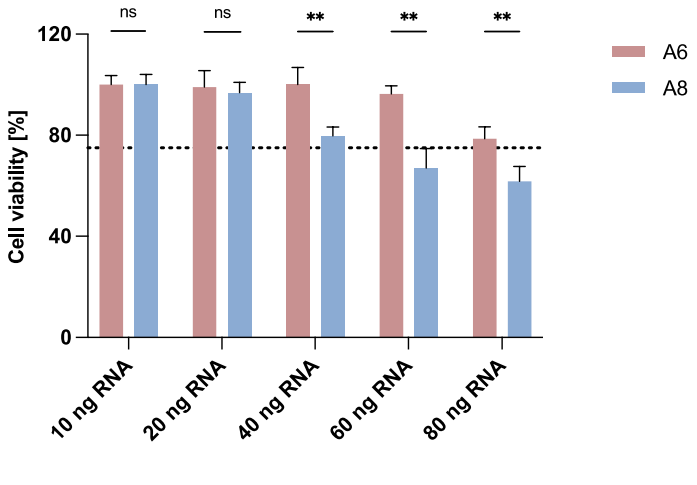
Gene editing efficiency in A549 cells



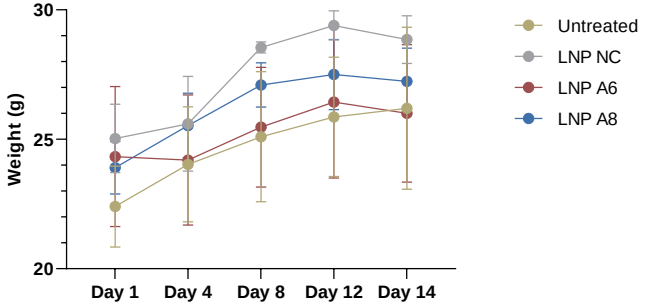
Cell apoptosis

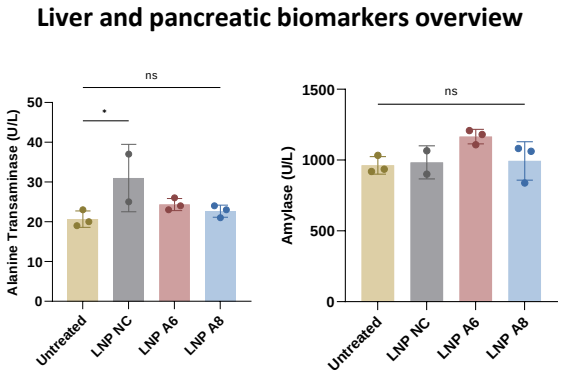


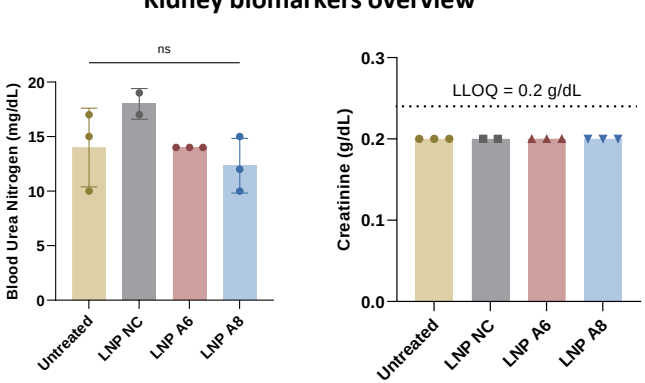
Cytotoxicity

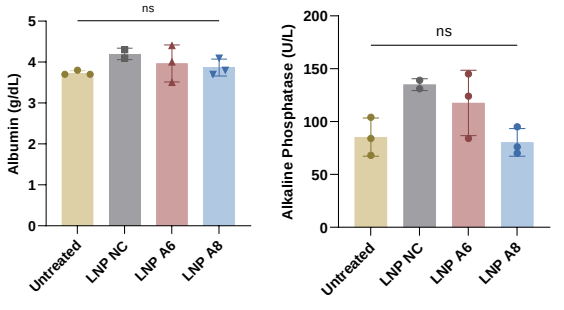


Gene editing evaluation in an animal model









- No signs of toxicity were reported after repeated administration of the best-performing formulations in healthy animals

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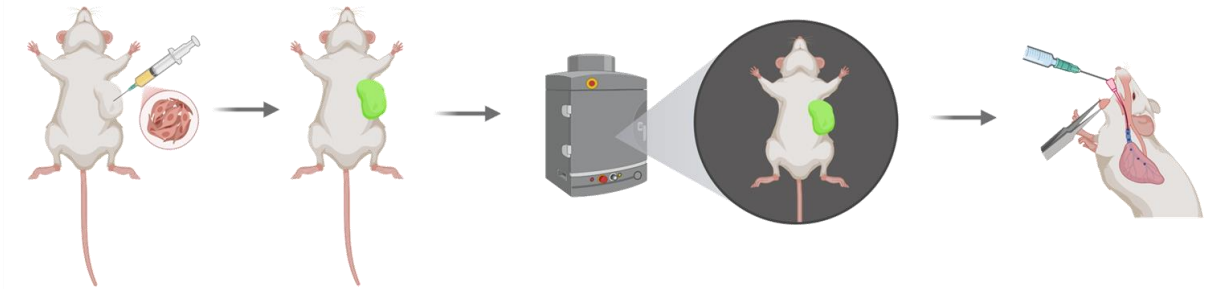
Conclusion and outlook

- Successful identification of two lead candidates ⇢ high gene editing percentages, apoptosis activity, low cytotoxicity, and no toxicity in animals

- Efficacy study (ongoing):
  - Goal: to evaluate the anti-tumor effect of experimental treatments using an orthotopic mouse model of lung cancer



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